7. Myrtill-agar. Each teaspoonful represents 1 c.c. (♏ 16) tinctura myrtill (U. S. P.).

8. Ipecacuanha-agar. Each teaspoonful represents 1 c.c. (♏ 16) tinctura ipecacuanhæ (U. S. P.).

9. Sumbul-agar. Each teaspoonful represents 1 c.c. (♏ 16) fluidextractum sumbul (U. S. P.).

Phenolphthalein and rhubarb-agar (1 and 2) can be conveniently used for the different varieties of constipation. One teaspoonful of either twice daily, after breakfast and supper, in some water, is the average dose. This, of course, may be increased or diminished according to the requirements of the case.

Calumba-agar (3) has proved valuable in cases of colitis (appearance of considerable mucus in the stool) with normal defecation. The average dose is one teaspoonful three times a day after meals, in some water.

Gambir-, tannin-, simaruba-, and myrtill-agar (4, 5, 6, and 7) I have found valuable in diarrheal conditions (acute and principally chronic). The average dose is usually one teaspoonful three times a day, after meals. Myrtill-agar I have also employed in diabetes mellitus, especially if complicated with enteritis.

Ipecacuanha-agar (8) I had prepared with the intention of administering it in amebic dysentery. I have, however, not yet tested it.

Sumbul-agar (9) I have employed in a considerable number of neurotic intestinal disturbances with seemingly good results. The dose was one teaspoonful three times daily after meals, in some water.

As a whole I can recommend the above various agar preparations as valuable remedies in intestinal therapeutics.

REACTIONS INDUCED BY ANTITYPHOID VACCINATION.

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With the object of determining the reactions induced by typhoid bacillus vaccines, we made a series of examinations of ten medical students, who voluntarily received the vaccination during the early part of the past year. We were especially interested in the change in the number and proportion of leukocytes, but made such other determinations as were necessary to demonstrate that specific antibodies were actually produced. Finding that better results were obtained with a vaccine prepared at the Army Medical School, Washington, D. C., than one which was prepared from our own culture, we used vaccines supplied by Major Russell. This vaccine (No. 44) was prepared by cultivating a non-virulent strain of the typhoid bacillus on slanted agar for eighteen hours, then making a suspension of the growth in sterile salt solution and diluting it so that each cubic centimeter contained 1,000,000,000 bacilli. This was then sterilized at a temperature of 56° C. for one hour and its sterility tested by aërobic and anaërobic cultivation and by animal inoculation. After the addition of tricresol (to make a 0.25 per cent. solution) as a preservative the vaccine was placed in sterile glass ampoules, which were sealed by heat.

Artificial immunization against typhoid fever was first tried on lower animals by means of living cultures of typhoid bacilli. Among the earlier experimenters were Simmons and Fränkel (1886), Beumer, Chantemesse, Widal and Sanarelli.[1] In 1892 Brieger, Kitasato, and Wassermann[2] determined that the use of killed typhoid bacilli was quite as efficient in inducing the production of antibodies as were living bacteria. Pfeiffer's[3] work in 1893 and the following year, in demonstrating the possibility of inducing marked bacteriolysis in the body (Pfeiffer's phenomenon) was a great stimulus to further research, as was also the work on agglutinins by Grubler and Durham in 1896. In 1896 Pfeiffer and Kolle,[4] working together, and A. E. Wright[5] independently, inoculated two men with typhoid vaccines. The laboratory evidence of the production of immunity was sufficiently great to warrant the application of the vaccination on a more extensive basis. In 1897 Wright[6] reported the inoculation of eighteen persons, and the following year introduced antityphoid vaccination on a rather large scale among the English soldiers engaged in the Boer war in southern Africa. Certain unfavorable reports regarding the work in southern Africa caused a partial suspension of the work for a few years. In 1904, on the advice of R. Koch, it was introduced in the German army, and a little later reintroduced among the English soldiers through the efforts of Col. Leishman. In 1908 it was introduced in our own army, the vaccine being prepared under the direction of Major Russel, of the Army Medical School in Washington. The submission of our soldiers to vaccination was at first voluntary but was made compulsory in 1911.

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[1] Handbuch der Technik und Methodik der Immunitätsforshung, i. 723.

[2] Zeitschr. f. Hyg., 1892, xii, 137.

[3] Deut. med. Woch., 1896, Nos. 7 and 8.

[4] Deut. med. Woch., 1896, p. 735.

[5] Lancet, September 19, 1896, p. 807.

[6] Brit. Med. Jour., January 30, 1897, p. 16.

Clinical Evidence of Protection. The statistics of Wright[7] on his vaccinations during the Boer war are based on 19,069 soldiers vaccinated as compared with 150,231 not vaccinated. Roughly considered, the vaccination reduced the incidence of the disease about 50 per cent. and the mortality almost 75 per cent. Later, with better technique in the preparation of the vaccine and having the vaccination consist of two or three injections, the results have been still better. According to a recent report of Leishman,[8] based on 5473 persons vaccinated as compared with 6610 not vaccinated, the number of cases of typhoid fever and deaths from that disease was only about one-tenth as high among the former as among the latter. Instances in which protection was definitely proved, although on a smaller scale, are quite numerous.

Our vaccinations consisted of three subcutaneous injections in the arm at intervals of ten days, the first dose consisting of 500,000,000 typhoid bacilli and the other two of 1,000,000,000 each. The reaction may be both local and systemic in character. The local reaction begins usually in from four to five hours after injection and gradually subsides in three to five days. At its height it usually consists of an area of redness about 10 cm. in diameter, which is swollen and tender. Occasionally the axillary lymph nodes are also slightly enlarged and tender. A systemic reaction, consisting of one or more of the following symptoms, pyrexia up to 103° F., headache, malaise, insomnia, nausea, and in rare instances backache, vomiting, chills, herpes labialis, loss of weight, and albuminuria, also sometimes develops. These general symptoms begin usually in five to six hours after injection and disappear before the lapse of forty-eight hours. The reactions following the different injections in the same individual may vary somewhat, but taken on an average they become progressively less (see Table I).

TABLE I.

	Local redness and swelling 8 cm. or more in diameter.	Pain or tenderness in axilla.	No increase of temperature.	Temperature from 98.6 to 100.	Temperature from 100 to 103.	Temperature 103 or above.	Headache	Backache.	Insomnia.	Nausea.	Chills.	Malaise.	"Soreness all over."
First injection . .	10	5	4	6	0	0	2	3	4	2	3	6	1
Second injection . .	10	2	3	7	0	0	1	1	0	0	0	2	3
Third injection (on basis of 9) . . .	9	0	2	7	0	0	0	0	0	0	0	0	0

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[7] Quoted by Russell, The Military Surgeon, 1909.

[8] Royal Army Medical Corps, 1909, xii, p. 166.

The frequency with which the signs and symptoms of the reaction developed in the individuals vaccinated, who were male students from twenty-one to thirty years of age, is given in the above table. Ten students received two injections, but only 9 were vaccinated a third time.

The mild character of the reaction is indicated by the fact that of more than 30,000 injections given to the soldiers of the United States Army, there was either no general reaction or only a mild one (temperature elevated but not up to 100° F., or merely headache and malaise) in about 95 per cent., and that in no instance were there any untoward results. In addition to the ten individuals of our series mentioned above, we also obtained a volunteer for vaccination who had had an attack of typhoid fever nine years previously. Following the first injection he had a severe local reaction consisting of swelling and redness of the entire upper arm and extending for two inches below the elbow. This inflamed area, which was very tender, continued for twenty-four hours. After his second injection the above-mentioned symptoms were even more marked and the individual had a temperature of 103.5° and was delirious for ten hours. In consideration of the severity of these reactions it was not thought advisable to give the usual third injection. Russell also reports more severe reactions in those who have previously had typhoid fever.

The reactions upon which the immunity depends or by which it may be measured may be divided into leukocytic, opsonic, phagocytic, agglutinic, and bacteriolytic. That the bacteriolysins are increased can easily be determined by the method of Pfeiffer. The bacteriolytic tests are, however, very unsatisfactory, inasmuch as no method has yet been devised by which the amount of bacteriolysins may be accurately determined. Although some observers[9] have demonstrated that there is a marked increase in the opsonins, determination of the opsonic index is rather unsatisfactory. Somewhat more satisfactory is the estimation of the power of phagocytosis by the dilution method of Neufelt. Using this method, Russell[10] has found that, beginning about the fifth to eighth day after the first injection, there was a rapid rise in the power of phagocytosis, reaching its height in about three weeks, when the phagocytic titer was one to two, occasionally five or six, thousand times as high as that of normal blood, after which it gradually declined until at the end of one year it was again normal. The agglutinins begin to increase at about the same time as the opsonins, as determined by the power of phagocytosis, and rise rapidly until, at the end of three or four weeks, it is possible to get agglutination (Widal reaction) with a dilution of blood serum 500 to 5000 times, or in some cases even 20,000 times. After this time it gradually declines, although it may still be possible to secure agglutination with a dilution of 1 to 100 at the end of a year.

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[9] Jour. of Med. Research, June, 1910, xxii (New Series, xvii), No. 3, p. 435.

[10] Boston Med. and Surg. Jour., January 5, 1911, clxiv, No. 1, p. 1.

Although a number of investigations relative to the degree of immunity as measured by the factors just mentioned have been made, we have found no record of a study of the leukocytic reaction following antityphoid vaccination in the human being. With the object of making such a study, careful leukocyte counts, both total and differential, were made of the ten subjects of our experiment. All of the counts were made at a time to avoid, so far as possible, the influence of digestion. Controls made by injecting as much salt solution and tricresol as is found in one dose of the vaccine did not give any leukocytic reaction.

Table II gives the average leukocyte count (total and differential) of the ten persons as determined before and at different times after the three injections.

Table II.

	Total number leukocytes.	Total and percentage of various types of leukocytes.									
		Polymorphonuclear neutrophiles.		Small lymphocytes.		Large mononuclears.		Eosinophiles.		Transitionals.	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
FIRST INJECTION.											
Just before	8400	5088	60.7	2544	30.0	424	5.0	137	2.8	132	2.7
One day after	10360	7148	69.3	2020	19.5	736	7.1	176	1.7	124	1.2
Two days after	8680	5900	67.6	1300	15.0	868	10.0	195	2.6	130	1.5
Three days after	8130	4376	61.2	2057	25.3	772	9.5	138	1.7	162	2.0
Four days after	7140	3806	58.3	2070	29.0	685	9.6	164	2.3	57	0.8
Five days after	7111	4338	61.0	1586	22.3	704	9.9	185	2.6	92	1.3
Six days after	7210	4297	59.6	1859	25.8	576	8.0	331	4.6	201	2.8
Seven days after	7960	5150	64.7	1926	24.2	562	7.1	127	1.6	166	2.1
SECOND INJECTION.											
Just before	6510	3647	54.8	2070	31.8	560	8.6	160	2.6	104	1.6
One hour after	7433	4159	57.3	2378	32.0	520	7.0	74	1.0	170	2.3
Six hours after	13540	9522	70.4	2978	22.0	947	7.0	121	0.8	119	0.8
One day after	9280	6958	71.0	1959	21.0	...	....	55	0.6		
Two days after	7911	5505	69.6	1379	17.4	688	8.7	63	0.8	158	2.0
Four days after	7000	4179	59.7	1799	25.7	749	10.7	98	1.4	98	1.4
Seven days after	6311	4077	59.0	2142	31.0	463	7.0	276	0.4	138	2.0
THIRD INJECTION.											
Just before	7742	4830	62.4	1455	18.8	836	10.8	155	2.0	155	2.0
One hour after	9600	6375	66.4	1920	22.4	499	5.2	192	1.2	132	2.0
Two days after	7400	4884	66.0	1659	22.4	444	6.0	59	0.8		
Four days after	6400	3667	57.3	1536	24.0	723	11.2	256	4.0	211	3.3

Chart I shows in a graphic way that the increase and decline in the total number of leukocytes occurs relatively about the same time following each injection. The degree of leukocytosis was highest following the first injection, and was successively less for the two subsequent injections. The curve following the second injection is the most representative one, inasmuch as several counts were made at short intervals following this injection. It indicates that the rise in the number of leukocytes in the peripheral blood is rapid and probably at its height in about six hours when the average count was 13,540 as compared with 6510 just before the injection. This corresponds to the time when the subjective symptoms are just beginning. The total count rapidly falls again, to reach the normal level on the second or third day after injection. In one instance the leukocyte count raised from 7200 just before injection to 8600 one hour later and 22,800 six hours after injection.

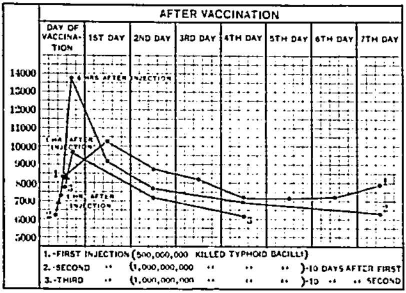

Chart I.—Total leukocyte count following antityphoid vaccination.

Chart II represents in a graphic way the total leukocyte count and the total number of each of the different types of leukocytes following the several injections. It will be noticed that the increase in the total number of leukocytes is due almost entirely to an increase in the number of the polymorphonuclear neutrophile leukocytes and the large mononuclears; the small lymphocytes the transitional cells and eosinophiles remaining about the same. It will also be noticed that the fall in the number of neutrophiles is more rapid than that of the total count, due to the fact that the large mononuclear counts remain rather high for from four to seven days after the injection.

Chart III represents graphically the percentage of the different types of leukocytes following the several injections. Considering the curves given in Chart II, it is very apparent that the fall in the percentage of small lymphocytes, due to a corresponding increase in the percentage of the neutrophiles during the first six hours after injection, caused a slight decrease in the percentage of the large mononuclear leukocytes. The percentage of the latter rose, however, to above normal, even while the percentage of the neutrophiles was still high. The percentage of the large mononuclears, moreover, continued to remain above the normal even after the total count had become normal.

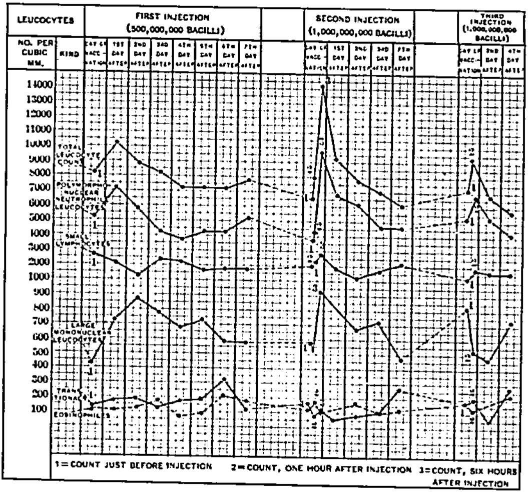

CHART II.—Leukocyte count, total and differential, following antityphoid vaccination.

It will be noted that there are two things about the leukocytic reactions that stand out prominently—(1) the high leukocytosis following injection of the vaccine, and (2) the considerable increase, both relative and absolute, in the number of large mononuclear leukocytes.

The increase in the total leukocyte count is of interest, inasmuch as there is usually no leukocytosis in uncomplicated cases of typhoid fever. It is due principally to an increase in the number of polymorphonuclear leukocytes. Such may readily be explained by the rather marked local reaction induced by the vaccine.

The increase in the number of large mononuclear leukocytes is, however, we believe, of more significance. The large mononuclear cells mentioned all had the same general appearance, but varied in size. A few of them were about the size of the polymorphonuclear leukocyte, but most of them were two to three times as large. The nucleus was usually slightly eccentric, but not peripheral, there being a thin ring of cytoplasm entirely around it. The diameter of the nucleus was usually about one-half the diameter of the whole cell. It was stained deeply blue, being of about the same intensity as the nucleus of the small lymphocytes. The cytoplasm was of a paler blue than the nucleus, but not purplish. There were no definite granules in the cytoplasm, but it was occasionally noted that there seemed to be a very slight fibrillar character to it. At times it was noted that the nucleus was rather indefinite in outline and merged imperceptibly into the surrounding cytoplasm. The stain used was eosinated or erythrosinated methylene blue.

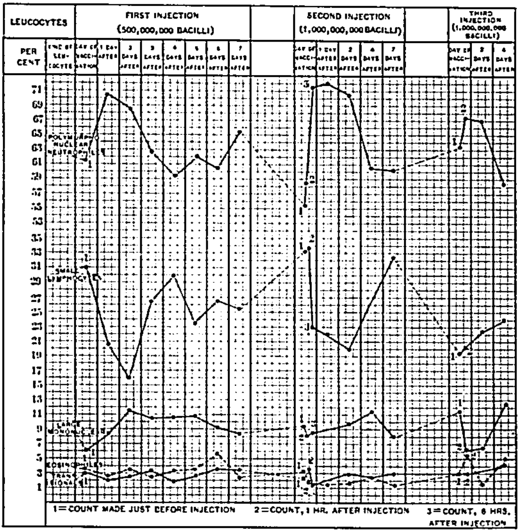

Chart III.—Differential leukocyte count following antityphoid vaccination.

Various observers have noted that there is an increase in the number of the large mononuclear leukocytes in typhoid fever and during convalescence from that disease. Thayer,[11] on analyzing the blood findings of 832 cases of uncomplicated typhoid fever, found that the large mononuclear leukocytes represented on an average 12.4 per cent. of the leukocytes during the first week of the disease, 14.4 per cent. during the fourth week, 16.8 per cent. during the eighth week, at about which percentage it remained until after the fifth week of convalescence.

De Sandro,[12] conducting certain experiments in fatigue with dogs, rabbits, and guinea-pigs, and using "typhoid toxin" as a test of resistance, found that, after injection, there was an initial leukopenia, followed by a leukocytosis, at first of the polymorphonuclear type and later of the mononuclear (especially large) type. Of importance is the observation that in the fatigued animals the initial leukopenia was more marked and prolonged and the subsequent leukocytosis (both polymorphonuclear and mononuclear) was less intense and persistent than in the normal controls. He also found that the influence of fatigue caused the agglutinins to develop less rapidly and to a less extent than under normal conditions.

SUMMARY AND CONCLUSIONS. 1. Statistics based on a large number of cases show conclusively that antityphoid vaccination confers a marked degree of protection against typhoid fever.

2. The injection of typhoid vaccines induces a local reaction in all cases and a general reaction in some cases.

3. A previous attack of typhoid fever apparently causes the reaction to be more severe than is observed in individuals who have not had typhoid fever.

4. Antityphoid vaccination causes a marked increase in the specific agglutinins, opsonins, and bacteriolysins.

5. The injection of typhoid vaccines causes a marked polymorphonuclear neutrophile and large mononuclear leukocytosis.

6. The marked increase (both absolute and relative) of the large mononuclear leukocytes in the peripheral blood is the only leukocytic change which is common to both clinical typhoid fever and antityphoid vaccination.

7. Such occurrences suggests that these leukocytes have something to do with the formation of antibodies concerned with the production of antityphoid immunity.

8. It seems well worth while to attempt experiments on the artificial production of large mononuclear leukocytosis and to see what relation such may have to the production of antibodies in the presence of typhoid infection or antityphoid vaccination.

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[11] Johns Hopkins Hosp. Rep., 1900, viii, 487.

[12] Riforma Med., 1910, xxxv, 841 to 871; abstract in Journal of Amer. Med Assoc., September 17, 1910, p. 1064.